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# Regulation of Protein Metabolism by a Physiological Concentration of Insulin in Mouse Soleus and Extensor Digitorum Longus Muscles

## EFFECTS OF STARVATION AND SCALD INJURY

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1. Although high concentrations of insulin affect both synthesis and degradation of skeletal-muscle protein, it is not known to what extent these effects occur with physiological concentrations. The effects of a physiological concentration of insulin (100 $\mu$units/ml) on muscle protein synthesis, measured with [$^3$H]tyrosine, and on muscle protein degradation, measured by tyrosine release in the presence of cycloheximide, were studied in mouse soleus and extensor digitorum longus muscles *in vitro*. 2. Insulin significantly stimulated protein synthesis in both muscles, but an inhibition of degradation was seen only in the extensor digitorum longus. 3. Starvation for 24h decreased the rate of protein synthesis and increased the rate of breakdown in the extensor digitorum longus. Sensitivity to insulin-stimulation of protein synthesis in the soleus was increased by starvation. 4. A 20%-surface-area full-skin-thickness dorsal scald injury produced a fall in total protein content in soleus and extensor digitorum longus muscles, maximal on the third day after injury. Soleus muscles 2 days after injury showed an impairment of protein synthesis; degradation was unaffected, and neither synthesis nor degradation *in vitro* was significantly affected in the extensor digitorum longus. 5. The advantages and limitations of studies of protein metabolism *in vitro* are discussed.

Skeletal-muscle protein plays an important role in whole-body metabolic homoeostasis (Ruderman, 1975; Daniel *et al.*, 1977). Insulin is thought to be one of the major regulators of muscle protein metabolism (Cahill *et al.*, 1972); loss of muscle protein is marked in untreated diabetes mellitus, and infusion of insulin *in vivo* suppresses amino acid release from muscle (Pozefsky *et al.*, 1969; Felig & Wahren, 1974).

The mechanism of regulation of protein metabolism by insulin has been widely studied *in vitro*. Insulin affects both synthesis and degradation of muscle protein (reviewed by Rannels *et al.*, 1977). However, it is not clear to what extent each of these effects might contribute to its effects *in vivo*. In early studies, insulin in a physiological concentration (50 $\mu$units/ml) was found to stimulate incorporation of labelled amino acids into protein in rat diaphragm (Manchester & Young, 1959; Manchester *et al.*, 1959). Increased incorporation of label, however, can arise from either a stimulation of synthesis or an inhibition of degradation, causing increased precursor-pool specific radioactivity. Although methods are now available for measuring synthesis and degradation of muscle protein independently, the question of the concentrations of insulin required has rarely been re-examined. Most workers have used extremely high insulin concentrations [e.g. 100 munits/ml (Wool & Manchester, 1962; Goldstein & Reddy, 1970; Fulks *et al.*, 1975); 25 munits/ml (Morgan *et al.*, 1972; Lundholm & Scherstén, 1977); 5–10 munits/ml (Adolfsson *et al.*, 1967; Short, 1969; Chain & Sender, 1973)], so the relevance of their results to the role of insulin *in vivo* is dubious. Rannels *et al.* (1975), however, found that only at concentrations of 200 $\mu$units/ml or more, well above its normal plasma concentration in the rat (e.g. Hoffmann *et al.*, 1972), did insulin significantly affect protein degradation in perfused rat heart, and Li *et al.* (1975) and Jefferson *et al.* (1977) found that a concentration of 500 $\mu$units/ml was needed to inhibit degradation in rat skeletal muscle.

This question has now been examined by using preparations of voluntary skeletal muscle *in vitro*. Rates of protein turnover (Goldberg, 1967; Laurent *et al.*, 1978) and their control by other hormones (Goldberg & Goodman, 1969; Tomas *et al.*, 1979) differ in different muscle types, and so the soleus and extensor digitorum longus muscles have been used as examples of predominantly red and white muscle respectively (Maizels *et al.*, 1977). Rather than carry out these experiments on young, rapidly growing rats, mice have been used; each of these muscles from a

nearly adult mouse is well below the size at which diffusion may become rate-limiting *in vitro* (Chaudry & Gould, 1968).

Against the background information obtained from these experiments, the effects of scald injury on muscle protein metabolism were also investigated. Loss of muscle protein after injury or operation has serious effects on recovery (reviewed by Kinney, 1977), and yet little is known of the mechanism involved in this response. There is debate about whether the major effect is an impairment of protein synthesis or an increase in protein degradation (e.g. Hinton & Allison, 1971; O'Keefe *et al.*, 1974; Brennan *et al.*, 1975; Crane *et al.*, 1977), and also on the part played by malnutrition (e.g. Flatt & Blackburn, 1974; Richards, 1977).

## Experimental

### *Animals*

Male albino mice weighing 22–35g were used. These were kept at 20°C with 12h light per day and fed on P.M. Diet (Oakes Millers Ltd., Congleton, Cheshire, U.K.). Unless otherwise stated, 2 days before the experiment each mouse had its dorsal fur clipped under ether anaesthesia (for comparability with those injured). Injury consisted of a 20%-body-surface-area full-skin-thickness scald injury produced at this time (Frayn *et al.*, 1978). Control and injured mice were fed *ad libitum*. Starvation consisted of food deprivation for 24h before killing.

### *Incubation methods*

Mice were killed by cervical dislocation and a blood sample was taken from the chest and rapidly centrifuged. The soleus (Cuendet *et al.*, 1976) and extensor digitorum longus muscles were removed from each leg by placing ties around their distal tendons, removing the overlying muscles, and then placing ties around the proximal tendons before removing the muscles and attaching under slight tension to stainless-steel wire holders for incubation. With two operators the time from killing to the beginning of incubation of all four muscles was about 5min. Each muscle was incubated at 37°C in a polystyrene tube (LP/3; Luckham Ltd., Burgess Hill, Sussex, U.K.) with constant gassing with $O_2/CO_2$ (19 : 1) via a fine polyethylene tube, with addition of a drop (approx. 5$\mu$l) of a silicone antifoam compound (Dow Corning Antifoam M Compound; Hopkin and Williams, Chadwell Heath, Essex, U.K.) (Frayn & Maycock, 1979). The incubation medium was 1.5ml of Krebs–Henseleit bicarbonate buffer (Dawson *et al.*, 1969) containing glucose and fatty acid-free albumin [Sigma (London) Chemical Co., Poole, Dorset, U.K.] (2mg/ml each) and the additions specified below. Insulin (bovine, 6× recrystallized; gift from Boots Co., Nottingham, U.K.) was added to the medium as required. All muscles were given 30min preincubation with all additions except labelled tyrosine, then placed in 1.5ml of fresh medium and incubated for the time specified.

Protein synthesis and degradation rates were measured as described by Fulks *et al.* (1975) and Goldspink (1976). Synthesis was measured by incubation with L-[*side-chain*-2,3-$^3$H]tyrosine (The Radiochemical Centre, Amersham, Bucks., U.K.) (approx. 1mCi/litre; 0.1mM). At the end of incubation the muscle was removed, blotted, frozen in liquid nitrogen and homogenized under liquid nitrogen by pounding with a glass rod in a polypropylene micro-centrifuge tube (1.5ml capacity) before adding 0.5ml of trichloroacetic acid (10%, w/v). After centrifugation, portions of the supernatant solution were used for tyrosine assay (250$\mu$l) and for determination of radioactivity (100$\mu$l). The pellet was washed twice with trichloroacetic acid (1.0ml; 5%, w/v) and once with ethanol/diethyl ether (1.0ml; 1 : 1, v/v) and then dissolved in NaOH (0.5ml; 1M). Portions of this solution were used for determination of protein (20$\mu$l) and of radioactivity (400$\mu$l). Portions of unused incubation medium were also kept for determination of radioactivity. The intracellular specific radioactivity of tyrosine was calculated (Fulks *et al.*, 1975) by using the extracellular spaces shown in Table 1, and the rate of tyrosine incorporation was calculated by using intracellular specific radioactivity as an estimate of precursor-pool specific radioactivity (Fern & Garlick, 1973). Although the values for the extracellular spaces were higher than those given by others (Sréter & Woo, 1963; Goldspink, 1976), the calculated rate of protein synthesis is very insensitive to the value used (Table 2).

Protein degradation was measured by incubation in a medium containing cycloheximide (0.5mM) and

Table 1. *Extracellular spaces in mouse soleus and extensor digitorum longus muscles*

Muscles were given 30min preincubation and then 60min incubation as described in the text in the presence of [U-$^{14}$C]sucrose (The Radiochemical Centre) (0.5mCi/litre; 1mM). They were then gently blotted and dissolved in NaOH (0.5ml, 1M) for determination of radioactivity and protein content. Results are in $\mu$l/mg of protein and are expressed as means ± S.E.M. (*n*). Significance of difference from controls (*t* test): *$P < 0.01$.

| Treatment | Soleus | Extensor digitorum longus |
|---|---|---|
| Control | 2.44 ± 0.11 (21) | 1.80 ± 0.16 (13) |
| Starved | 2.29 ± 0.26 (6) | 2.12 ± 0.25 (6) |
| Scalded | 1.97 ± 0.12 (18)* | 1.53 ± 0.11 (19) |

Table 2. *Insensitivity of calculated rate of protein synthesis to value used for extracellular space*
Rates of protein synthesis (μmol of tyrosine/g of protein in 3h) were calculated as described in the text by using data for two typical soleus and two extensor digitorum longus muscles from control mice. The values for extracellular space used in the calculation were varied as shown.

| Extracellular space (μl/mg of protein) ... | | 3 | 2.44 | 2 | 1.8 | 1 | 0.2 |
|---|---|---|---|---|---|---|---|
| Soleus | 1 | 2.09 | 1.93 | 1.83 | 1.79 | 1.65 | 1.55 |
| | 2 | 1.84 | 1.71 | 1.63 | 1.59 | 1.48 | 1.40 |
| Extensor digitorum longus | 1 | 1.12 | 1.04 | 1.00 | 0.98 | 0.91 | 0.87 |
| | 2 | 0.88 | 0.83 | 0.80 | 0.78 | 0.74 | 0.71 |

no added tyrosine, and measuring tyrosine release into the medium. Cycloheximide decreased protein synthesis by over 99% (exact calculation is difficult, since the relevant intracellular specific radioactivity is uncertain). After incubation the muscles were blotted and dissolved in NaOH (0.5ml, 1M) for protein determination, and portions of the incubation medium (250μl) used for tyrosine determination.

### *Analytical methods*

Concentrations of plasma glucose were determined by using hexokinase (Schmidt, 1961) and of plasma insulin by using a charcoal-separation radioimmunoassay (Herbert *et al.*, 1965) with mouse insulin standards (Novo Research Institute, Bagsvaerd, Denmark). Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as the standard. Tyrosine was measured by the method of Waalkes & Udenfriend (1957) by using kit 70-F from Sigma. Radioactivity was determined in 10ml of Instagel (Packard Instrument Co., Downers Grove, IL, U.S.A.), with correction for efficiency by using the external-standards ratio.

### *Experimental design and statistical methods*

Wherever possible the effects of additions to the incubation medium were tested 'within animals' (i.e. the muscles from one leg used as controls) and significances calculated by using the paired *t* test. Statistical methods were those described by Snedecor & Cochran (1967).

## Results

### *Incubation conditions*

Optimal incubation conditions were investigated before commencing the main part of the study. For these experiments tyrosine incorporation was calculated based on specific radioactivity in the medium (rather than intracellular), and so no conclusions can be drawn about mechanisms involved. The time-course for incorporation of label (Fig. 1) was similar to that observed by Morgan *et al.* (1972) and by Jefferson *et al.* (1974) in perfused rat heart and psoas muscle, remaining almost linear for 3h in the presence of insulin. All further experiments were done with incubation for 3h. The effects of addition of branched-chain and of all amino acids at approximately their plasma concentrations (Morgan *et al.*, 1972) were tested (Table 3). Branched-chain amino acids

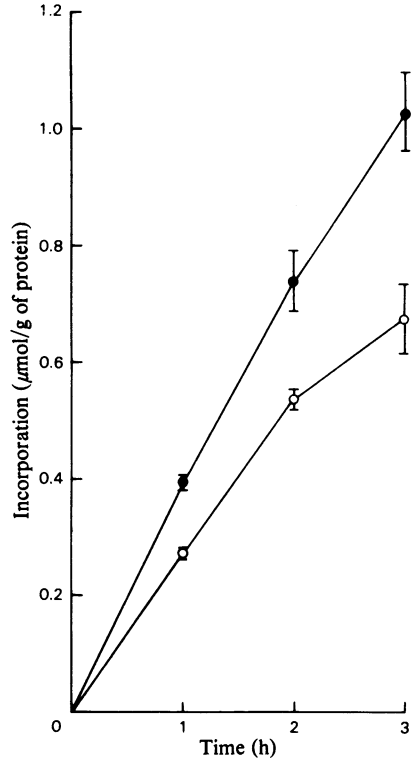


Fig. 1. *Effect of insulin on incorporation of [$^3$H]tyrosine into protein in mouse soleus muscle in vitro*
Soleus muscles were given 30min preincubation, then incubated with L-[*side-chain*-2,3-$^3$H]tyrosine as described in the text. Incubation was stopped at the times shown and tyrosine incorporation into protein calculated on the basis of specific radioactivity in the incubation medium. Symbols: ○, control; ●, insulin present (1munit/ml). Results are shown as means± S.E.M.; $n = 4$ per point.

significantly increased incorporation of labelled tyrosine in both the presence and the absence of insulin, but addition of all plasma amino acids had no further effect, as observed in rat diaphragm (Fulks *et al.*, 1975). All further studies of synthesis and degradation were therefore made with branched-chain amino acids present in the medium (leucine, 0.2mM; isoleucine, 0.1mM; valine, 0.2mM).

*Protein synthesis* (*Table* 4)

In muscles from control animals, rates of protein synthesis in the soleus were about twice those in the extensor digitorum longus. The rate of protein synthesis *in vitro* was decreased in the extensor digitorum longus by starvation for 24h, but was unaffected in the soleus. There was a systematic tendency for the rate of protein synthesis in the soleus to be correlated with that in the extensor digitorum longus from the same animal; this reached significance in individual groups only in muscles from starved mice incubated with insulin ($n = 8$, $r = 0.75$, $P < 0.05$) and in muscles from fed mice in the absence of insulin ($n = 25$, $r = 0.44$, $P < 0.05$).

Insulin (100$\mu$units/ml) caused a significant stimulation of synthesis in each muscle in all groups. The percentage increase in protein synthesis produced by insulin in muscles from control animals was similar in soleus and extensor digitorum longus muscles (Table 5). It was significantly increased in the soleus by 24h starvation.

Table 5. *Stimulation by insulin of protein synthesis in mouse soleus and extensor digitorum longus muscles in vitro*

Results are percentage stimulation by insulin within muscles from one animal, expressed as means±S.E.M. (*n*). Significance of difference from controls (Wilcoxon rank sum test) †$P$=0.02; significance of insulin-stimulation (paired *t* test) *$P < 0.05$, **$P < 0.02$, ***$P < 0.001$.

| | Stimulation (%) | |
|---|---|---|
| Treatment | Soleus | Extensor digitorum longus |
| Control | 36±10 (19)*** | 25±11 (20)* |
| Starved | 64±10 (8)†*** | 47±23 (9)* |
| Scalded | 32±13 (25)** | 30±9 (26)** |

Table 3. *Effects of amino acids and insulin on incorporation of [$^3H$]tyrosine into protein in mouse soleus and extensor digitorum longus muscles in vitro*

Muscles were incubated as described in the text. Branched-chain amino acids alone or all plasma amino acids were added to the medium at approximately normal plasma concentrations (Morgan *et al.*, 1972) as indicated. Results are $\mu$mol of tyrosine incorporated/g of protein in 3h incubation, based on specific radioactivity in the medium, and are expressed as means±S.E.M. (*n*). Significance of differences: insulin-stimulation (versus no insulin; unpaired *t* test) *$P < 0.02$, **$P < 0.01$; effect of branched-chain amino acids versus none (paired *t* test, $n = 20$) $P < 0.05$.

| Amino acids | Insulin ($\mu$units/ml) ... 0 | 100 | 1000 |
|---|---|---|---|
| None | 0.69±0.03 (10) | 0.81±0.04 (10)* | 0.91±0.06 (10)** |
| Branched-chain | 0.75±0.03 (8) | 0.84±0.06 (8) | 0.97±0.04 (8) |
| All | — | — | 0.96±0.04 (8) |

Table 4. *Rates of protein synthesis and ratio of intracellular specific radioactivity to that of the medium in mouse soleus and extensor digitorum longus muscles in vitro*

Muscles were incubated for 3h with L-[*side-chain*-2,3-$^3$H]tyrosine as described in the text. The rate of protein synthesis was calculated from the intracellular specific radioactivity. The ratio of intracellular specific radioactivity to that in the medium is also shown. Results are means±S.E.M. (*n*). For significance of effects of insulin on rates of synthesis, see Table 5. Significance of difference from corresponding control group (unpaired *t* test) *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; significance of effect of insulin on specific radioactivity ratio (paired *t* test) †$P < 0.02$.

| | Soleus | | Extensor digitorum longus | |
|---|---|---|---|---|
| Insulin ($\mu$units/ml) ... | 0 | 100 | 0 | 100 |
| Protein synthesis ($\mu$mol of tyrosine/g of protein in 3h) | | | | |
| Control | 1.329±0.096 (19) | 1.698±0.109 (19) | 0.693±0.038 (20) | 0.826±0.060 (20) |
| Starved | 1.033±0.045 (9) | 1.728±0.114 (8) | 0.406±0.041 (9)*** | 0.573±0.083 (9)* |
| Scalded | 1.113±0.094 (25) | 1.283±0.090 (27)** | 0.584±0.036 (28)* | 0.704±0.040 (26) |
| Specific-radioactivity ratio | | | | |
| Control | 0.56±0.05 (20) | 0.49±0.02 (20) | 0.60±0.03 (20) | 0.55±0.02 (21) |
| Starved | 0.58±0.04 (9) | 0.47±0.04 (8)† | 0.51±0.07 (9) | 0.54±0.04 (9) |
| Scalded | 0.60±0.04 (25) | 0.61±0.03 (27)** | 0.62±0.02 (28) | 0.62±0.03 (26) |

Rates of protein synthesis were, as expected, greater than rates of incorporation of tyrosine based on specific radioactivity of the medium (cf. Tables 3 and 4), because the intracellular specific radioactivity was lower than that in the medium (Table 4). Insulin did not systematically affect the ratio of intracellular specific radioactivity to that of the medium, despite its significant effects on protein breakdown (see below), although a significant decrease was observed in soleus muscles from starved mice incubated with insulin compared with the contralateral muscles in its absence. No differences in this ratio were seen between muscles from control and starved mice.

*Protein degradation* (*Table* 6)

Rates of protein degradation were higher than those of synthesis and, as for synthesis, were higher in the soleus than in the extensor digitorum longus. Starvation for 24h increased rates of protein breakdown *in vitro* in the extensor digitorum longus only, both in the presence and in the absence of insulin. Insulin caused significant inhibition of tyrosine release in the extensor digitorum longus only; in no group was there a significant effect in the soleus.

*Effects of scald injury*

Some acute effects of this form of scald injury have been described elsewhere (Frayn *et al.*, 1978). Mortality from injury in these experiments was 10% (9/86), similar to that observed previously (Frayn *et al.*, 1978), all deaths but one occurring within 24h of injury. The total protein content of soleus and extensor digitorum longus muscles fell significantly between days 1 and 3 after scalding (Table 7), a response very like that seen in the rat after similar injury (Threlfall *et al.*, 1979). All incubation experiments were therefore performed on day 2 after scalding, when it seemed that rates of change of muscle protein would be maximal.

Protein synthesis in soleus muscles from scalded mice (incubated with insulin) was decreased compared with fed controls (Table 4), although there was no significant effect in the extensor digitorum longus incubated with insulin. Stimulation of protein synthesis by insulin was maintained after injury (Table 5). The ratio of intracellular specific radioactivity to that of the medium was increased in the soleus incubated with insulin compared with muscles from fed or starved uninjured animals. Protein degradation *in vitro* was not affected (Table 6), and nor was its responsiveness to insulin, which, as in other groups, was observed in the extensor digitorum longus only.

*Plasma glucose and insulin concentrations* (*Table* 8)

Rates of protein synthesis *in vitro* were not correlated within any group with the plasma concentrations of glucose or insulin.

Table 6. *Rates of protein degradation in mouse soleus and extensor digitorum longus muscles in vitro*
Muscles were incubated for 3h and protein degradation was measured from the rate of release of tyrosine in the presence of cycloheximide (0.5mM) as described in the text. Rates are in μmol of tyrosine released/g of protein in 3h and all results are expressed as means±S.E.M. Inhibition by insulin was determined within animals. Significance of difference from corresponding controls (unpaired *t* test) $*P < 0.01$, $**P < 0.001$; significance of insulin-inhibition (paired *t* test) $\dagger P < 0.05$, $\dagger\dagger P < 0.002$.

| | *n* | Soleus | | | Extensor digitorum longus | | |
|---|---|---|---|---|---|---|---|
| | | Rate | | Inhibition | Rate | | Inhibition |
| Insulin (μunits/ml) ... | | 0 | 100 | (%) | 0 | 100 | (%) |
| Control | 21–24 | 4.29±0.25 | 3.88±0.17 | 6±6 | 2.54±0.10 | 2.22±0.16 | 17±5†† |
| Starved | 15–18 | 4.04±0.14 | 4.14±0.23 | 0±6 | 3.36±0.21** | 2.92±0.18* | 14±8† |
| Scalded | 13–14 | 3.70±0.26 | 3.71±0.25 | −3±8 | 2.26±0.12 | 1.90±0.09 | 13±3†† |

Table 7. *Effect of 20%-surface-area scald injury on protein content of mouse soleus and extensor digitorum longus muscle*
Soleus and extensor digitorum longus muscles were isolated intact at the times shown after 20% scald injury. The total protein content of each muscle was measured and is expressed relative to body weight immediately before injury, in mg of protein/kg body wt. Results are means±S.E.M.; five or six mice per group. Difference between days 1 and 3 was significant (analysis of variance) at $P < 0.025$.

| Time after injury (days) ... | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Soleus | 35.7±1.9 | 34.2±1.4 | 28.9±2.5 | 33.3±2.2 |
| Extensor digitorum longus | 56.0±1.8 | 51.5±2.7 | 50.5±3.2 | 55.0±1.8 |

Table 8. *Plasma glucose and insulin concentrations in control, starved and scalded mice*

Blood was taken from mice killed for preparation of muscles and plasma glucose and insulin concentrations were assayed as described in the text. Results are means±S.E.M. (*n*). Significance of differences from controls (*t* test): $*P < 0.01$, $**P < 0.001$.

| Treatment | Glucose (mM) | Insulin ($\mu$units/ml) |
|---|---|---|
| Control | 11.4±0.3 (26) | 87±6 (26) |
| Starved | 5.4±0.2 (12)** | 27±2 (12)** |
| Scalded | 10.1±0.3 (38)* | 91±13 (38) |

The plasma glucose concentration was halved, and the plasma insulin concentration decreased by about two-thirds, after 24h starvation. In contrast, 2 days after scald injury there was only a slight decrease in the plasma glucose concentration, and the plasma insulin concentration was normal. A small decrease in plasma glucose concentration has previously been noted at 24h after this injury (Frayn *et al.*, 1978) and is probably due to a lowered food intake.

## Discussion

Although studies of protein metabolism *in vitro* are necessary to describe the effects of insulin in mechanistic terms, and especially to investigate differences between different tissues, such studies have their limitations. The muscles in these experiments were in negative protein balance, as found in similar studies of rat muscles by Goldspink (1976) and by Li & Goldberg (1976). It appears that some trophic component of plasma is missing from the media used *in vitro*; when stimulation of incorporation of labelled glycine into rat diaphragm protein was used as a bioassay, the effects of two human plasma samples were equivalent to those of insulin concentrations of 2 and 10munits/ml (Manchester *et al.*, 1959), much greater than could have been accounted for solely by their contents of insulin. Positive nitrogen balance *in vitro* can be restored by addition of amino acids at five times normal plasma concentrations together with insulin at 10munits/ml (Goldspink, 1978), these conditions both increasing the rate of synthesis and decreasing that of breakdown, but this precludes investigation of the effects of lower concentrations of insulin. It has, however, been found (Goldspink, 1978) that comparative studies of muscle protein metabolism show similar differences between different groups of animals whatever the incubation conditions.

Comparison of rates of protein turnover measured in the present experiments with those *in vivo* is difficult, since the latter do not appear to have been measured in the mouse. Assuming the tyrosine content of muscle protein to be 3.5% (Turner & Garlick, 1974), the measured rates (in the soleus from control animals, incubated with insulin) correspond to fractional rates of synthesis and breakdown of about 5%/day and 12%/day respectively, as compared with a replacement rate of 9%/day in the adult rat soleus *in vivo* (Millward & Waterlow, 1978). For the extensor digitorum longus the corresponding values from the present experiments are about 2.5%/day and 7%/day for synthesis and breakdown respectively as compared with a replacement rate of 5%/day in the adult rat plantaris, a similar pale muscle.

These studies showed that insulin, in a physiological concentration for the mouse (cf. Table 8), significantly stimulated protein synthesis in both soleus and extensor digitorum longus. The effect of insulin at this concentration on protein breakdown, however, was seen only in the extensor digitorum longus. This difference in sensitivity between red and white muscles may explain why higher concentrations of insulin were needed to inhibit protein breakdown in perfused rat heart (Rannels *et al.*, 1975), an extreme example of redness, and in rat hemicorpus (Jefferson *et al.*, 1977), about half the mass of which consists of red fibres (Maizels *et al.*, 1977).

The effects of starvation on protein synthesis and degradation were greater in the extensor digitorum longus than in the soleus, as found both *in vivo* and *in vitro* by Li & Goldberg (1976). Since the rate of whole-body protein breakdown is probably decreased in early starvation (reviewed by Millward & Waterlow, 1978), white muscle may be an unusual tissue in demonstrating an increased rate of degradation. The decrease in protein synthesis and increase in degradation observed in the extensor digitorum longus *in vitro* presumably represent changes in the enzymic machinery of protein metabolism. A falling plasma insulin concentration is the main signal for the breakdown of muscle protein which occurs in early starvation (Ruderman, 1975), and this would be consistent with the observed changes, insulin affecting both synthesis and degradation in the extensor digitorum longus.

In the soleus, protein synthesis in the presence of insulin *in vitro* was unaffected by starvation. However, since the plasma insulin concentration was decreased so markedly (Table 8), a truer reflection of rates of synthesis *in vivo* is probably given by comparing *in vitro* the rate observed in the presence of insulin with muscles from fed mice (mean 1.70$\mu$mol of tyrosine/g of protein in 3h) and in its absence in muscles from starved mice (mean 1.03), the difference of 40% being significant (*t* test: $P < 0.001$). The increased responsiveness to insulin of protein synthesis in the soleus muscle after starvation might perhaps reflect the increase in insulin-receptor concentration that occurs in this muscle on starvation (Le Marchand-Brustel, 1978); although there is as yet no

evidence that the direct effects of insulin on protein synthesis are mediated through these receptors, its effect on amino acid transport is (Goldfine *et al.*, 1972), and this effect of insulin contributes a large proportion of its overall stimulation of protein synthesis (Goldstein & Reddy, 1970) as measured in these experiments.

Although it has been recognized for many years that loss of muscle protein is an important consequence of physical injury (Cuthbertson, 1964), the mechanism of this response has not received much study. Studies of protein turnover *in vivo* in man (O'Keefe *et al.*, 1974; Crane *et al.*, 1977) and rat (Hoover-Plow & Clifford, 1978) have shown that the major effect of surgical trauma is to decrease protein synthesis rather than to increase the rate of breakdown. After accidental injury, however, muscle protein breakdown may also be increased, as shown by increased excretion of $N^{\tau}$-methylhistidine (Williamson *et al.*, 1977; Bilmazes *et al.*, 1978). It seemed also that the response might differ in different types of muscle, particularly since there are differences in their response to hormonal control of protein metabolism (e.g. Goldberg & Goodman, 1969). The response to 20% scald injury in the mouse consisted of a short-lived but significant fall in total muscle protein content, more marked in the soleus than in the extensor digitorum longus. The only significant alteration to appear *in vitro* was a decrease of about 25% in the rate of protein synthesis in the soleus. If the protein turnover rate in this muscle *in vivo* is about 9%, as it is in the adult rat (Millward & Waterlow, 1978), this impairment of protein synthesis could contribute at least the major part of the response. After scald injury in the rat, however, $N^{\tau}$-methylhistidine excretion was increased during the period of net loss of muscle protein (Threlfall *et al.*, 1979), indicating an increased rate of muscle protein breakdown (Young & Munro, 1978). The reason for not observing any increase in the rate of breakdown *in vitro* in the present experiments might be either that the injury was slightly less severe (20% surface area against 30% in the rat) or that changes occurring *in vivo* were not manifest *in vitro*. Only certain sorts of changes will be carried over in this way; for instance changes in muscle glucose metabolism *in vivo*, presumably mediated by circulating hormones, occurring during the early phase of the response to injury, are not seen *in vitro* (Frayn *et al.*, 1978). Most hormonal effects on muscle protein breakdown, however, are thought to be mediated through changes in activities of intracellular enzymes (e.g. Mayer *et al.*, 1976; Röthig *et al.*, 1978; reviewed by Rannels *et al.*, 1977). It is unlikely that 2 days is simply too short a time for such changes to be established, since even 24h of starvation produced significant changes in both synthesis and breakdown *in vitro*.

The response to injury did not appear to depend to any great extent on malnutrition. There was a qualitative difference from the effects of starvation, in that the latter affected protein metabolism mainly in the extensor digitorum longus, whereas the effects of injury were more pronounced in the soleus. In addition, the injured animals were eating almost normally as judged by weight change (results not shown) and by their plasma glucose and insulin concentrations.

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